

functions of a strong constitutive promoter present in the said insulator construct following integration into the genome of a plant, said insulator construct comprising:

i) first transcription unit comprising a lethal gene under transcriptional control of a tissue specific promoter for targeted expression in specific tissue(s) and fused to a suitable transcription termination signal, comprising a polyadenylation signal,

ii) second transcription unit comprising a selectable marker gene under transcriptional control of a strong constitutive promoter and fused to a suitable transcription termination signal, comprising a polyadenylation signal, and

iii) an insulator sequence which is about 5kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements placed between the first and second transcription units so as to isolate the first transcription unit from enhancing influences of the constitutively expressing promoter in the second transcription unit,

wherein the insulator sequence functions in the absence of any inhibitor or protein in the background.

3. (Twice Amended) The construct as claimed in claim 1 wherein the lethal gene is selected from the group consisting of barnase gene, *RnaseTI* gene, *binase* gene, *rolB* gene, *rolC* gene and diphtheria toxin A gene.

4. (Twice Amended) The construct as claimed in claim 1 wherein the lethal gene is *barnase* gene.

5. (Amended) A construct as claimed in claim 1 wherein the tissue specific promoter of first transcription unit is selected from the group consisting of TA29, A9, A3, *tap1*, *bcpl*, and *napin*.

6. (Twice Amended) The construct as claimed in claim 1 wherein the insulator sequence is about 5kb in length and comprises coding sequences of *topoisomerase* gene from pea and *acetolactate synthase* gene from *Arabidopsis*.

11. (Twice Amended) The construct as claimed in claim 10 wherein the insulator sequence has the following properties:

(a) the insulator sequence does not encode any regulatory components or possess any enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content which is in consonance with transcriptionally active regions of a host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid

induction of Homology dependent gene silencing.

14. (Amended) The plant as claimed in claim 13 which is selected from the group consisting of a dicotyledonous and a monocotyledonous plant.

16. (Twice Amended) A method to obtain male-sterile plants in *Brassica juncea*, said method comprising the steps of:

i) transforming the nuclear genome of plant cells with a foreign DNA comprising:

a) a first transcription unit comprising a lethal gene under transcriptional control of a tissue specific promoter for targeted expression in specific tissue(s) and fused to a suitable transcription termination signal, comprising a polyadenylation signal,

b) a second transcriptional unit comprising a selectable marker gene under transcriptional control of a strong constitutive promoter and fused to a suitable transcription termination signal, comprising a polyadenylation signal, and

c) an insulator sequence which is about 5kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements placed between the first and second transcription units, so as to isolate the first

transcription unit from enhancing influences of the constitutively expressing promoter in the second transcription unit;

- ii) regenerating plants from said transformed plant cells,
- iii) identifying male sterile transgenic plants by the absence of pollen production and by their failure to set seed on selfing,
- iv) obtaining, at a high frequency, male sterile plants with normal vegetative morphology and normal female fertility,
- v) identifying single copy male sterile lines by Southern hybridization,
- vi) back-crossing male sterile plants with untransformed parent to obtain T1 seeds,
- vii) obtaining male sterile plants with normal T1 seed germination frequencies,
- viii) obtaining normal segregation ratio of the marker gene among T1 progeny of single copy male sterile plants identified,
- ix) transferring the marker gene containing T1 progeny to the field, and
- x) identifying the male sterile phenotype among all T1 progeny exhibiting marker resistance.

23. (Twice Amended) A method as claimed in claim 16 wherein male sterile lines in *Brassica juncea* are generated by

*Agrobacterium*-mediated transformation using disarmed Ti plasmid.

26. (Twice Amended) A method as claimed in claim 16 wherein T1 seeds are tested for their viability as evidenced by their ability to germinate on non-selective media.

27. (Twice Amended) A method as claimed in claim 16 wherein germinated T1 seedlings obtained from backcrossed seeds are tested for segregation of the marker gene by transferring them onto selective media.

30. (Amended) A method as claimed in claim 16, wherein the insulator sequence comprises coding sequences of *topoisomerase* gene from pea and *acetolactate synthase* gene from *Arabidopsis*.

31. (Amended) A method as claimed in claim 16, wherein the insulator sequence comprises the following properties:

(a) the insulator sequence does not encode any regulatory components or possess any enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content which is in consonance with transcriptionally active regions of a host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

Serial No.: 09/633,274

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing.

Please add the following claim:

32. The construct as claimed in claim 1 wherein the specific tissue(s) is tapetum.